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Journal of Electrocardiology 38 (2005) 45–50

JOURNAL OF Electrocardiology

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Electrical coupling of fibroblasts and myocytes: relevance for cardiac propagation

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Received 10 June 2005; accepted 10 June 2005

Abstract Myocytes, while giving rise to the bulk volume of normal cardiac muscle, form a "minority cell population" in the heart compared with nonmyocytes, chiefly fibroblasts. The heterogeneous cell types show very intimate spatial interrelation in situ, with virtually every myocyte in the mammalian heart bordering to 1 or more fibroblasts. Nonetheless, gap junction coupling in the heart is traditionally assumed to occur exclusively between myocytes. Yet, both freshly isolated cells and cell cultures have unambiguously shown functional heterogeneous myocyte-fibroblast coupling (mainly via connexin 43). Such coupling is sufficient, in vitro, to synchronize spontaneous beating in distant myocytes, connected over distances of up to 300 μm by fibroblasts only. More recently, functional myocyte-fibroblast coupling (via connexin 45) has been demonstrated in situ for sinoatrial node pacemaker tissue, and preliminary immunohistochemical data suggest that myocyte-fibroblast coupling may be present in postinfarct scar tissue. The functional relevance of such heterogeneous coupling for cardiac electrophysiology is only starting to emerge and has thus far mainly been assessed in theoretical studies. According to this research, fibroblasts may affect the origin and spread of excitation in several ways above and beyond formation of "passive" barriers that obstruct electrical conduction. Thus, fibroblasts may act as current sinks, contributing to the formation of unidirectional block or to the delay in atrioventricular conduction. Via short-range interaction, fibroblasts may help to smooth out propagating wave fronts, in particular in the sinoatrial node and in the cross-sheet direction of healthy ventricular myocardium, 2 tissues that might otherwise be expected to show fragmented conduction patterns. As long-distance communication lines, fibroblasts may bridge posttransplantation or ischemic scar tissue, with beneficial or detrimental effects on organ function (depending on the relation to normal conduction patterns), and explain the recruitment of myocyte islands embedded in fibrotic scar tissue. The inherent mechanosensitivity of cardiac fibroblasts could, furthermore, allow them to play a sensory role and to affect cardiac electrophysiology via mechanoelectric feedback. This article reviews the currently available experimental and theoretical evidence on the previous scenarios, and highlights areas for further research.

Keywords: Heart; Heterogeneity; Gap junction; Lucifer yellow; Mathematical model

1. Coupling of cardiac fibroblasts and myocytes

1.1. Histologic interrelation

Cardiomyocytes occupy most normal myocardial tissue volume, but they are the minority in terms of cell numbers. In the human heart, for example, myocytes account for only half of the cells at birth, and this share drops to about one third within 2 months of postnatal development, mainly because of continued fibroblast proliferation [1].

Fibroblasts are the dominant population among cardiac nonmyocytes. They are arranged in sheets and strands that run in parallel to the prevailing direction of muscle fibers or bridge "gaps" between cell groups or layers of myocardium. In essence, every cardiac myocyte is

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0022-0736/$ – see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.jelectrocard.2005.06.096

therefore in direct contact to 1 or more fibroblasts in situ (see Fig. 1A and B) [2].

The significance of the intimate interrelation of cardiac myocytes and fibroblasts is not yet fully understood. Clearly, many aspects of the integrated mechanical and humoral signaling between the homo- and heterogeneous cells benefit from spatial proximity (reviewed in detail elsewhere) [2-4]. In as much as cardiac electrical signaling is concerned, the classic view has been that connective tissue acts as an insulator, which, following upon disease-induced proliferation, may produce passive obstacles for conduction, thereby increasing cardiac electrical heterogeneity and contributing to arrhythmogenesis. The generality of this concept has been challenged in recent years, and the following sections illustrate the basis on which this challenge has evolved.

1.2. Fibroblast-myocyte coupling in vitro

Isolated cardiomyocytes and fibroblasts form functional gap junctional channels [5]. In cell cultures, these channels give rise to electrical contacts that are sufficient to allow fibroblast-based conduction of excitation, causing entrainment of rhythm in separate cardiomyocytes interconnected solely by fibroblasts [6]. The distances bridged by fibroblasts in vitro can be significant: up to 300 μm have been reported [7]. Of course, in vitro models offer only a rough

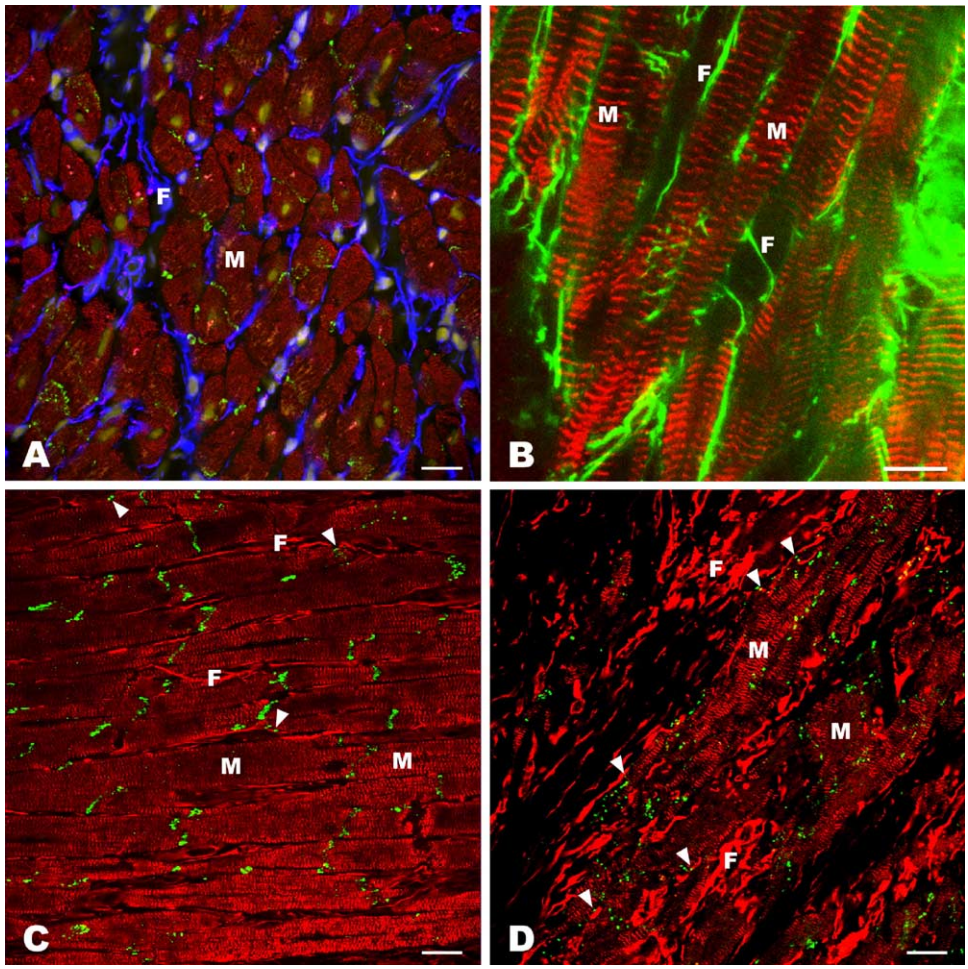

Fig. 1. Fibroblast myocyte interrelation in the heart. A, Confocal microscopy cross section of sheep ventricular myocardium, immunostained with antimyomesin to mark myocytes (red), antivimentin to mark fibroblasts (blue), anticonnexin 43 (bright green dots), and DAPI (4′-6-diamidino-2-phenylindole) to label nuclei (pale yellow-green patches), showing the dense network of fibroblasts that surrounds myocyte clusters of 2 to 4 cells (from Reference [2]). B, Lucifer yellow dye spread through rabbit right atrial fibroblasts (green) illustrates fibroblast coalignment with, and bridging of, gaps between cardiomyocytes, labeled with antimyomesin (red; from Reference [8]). C and D, Interrelation of Cx43 and cell types in sheep healthy ventricular tissue (C) and infarct border zone (D), revealed by triple-labeling for Cx43 (green), myomesin (red striated myocytes), and vimentin (solidly red fibroblasts). In healthy myocardium, Cx43 is largely organized in intercalated disks between myocytes but is also found as dispersed punctate labeling, some of which appears located at points of contact between myocytes and fibroblasts (arrowheads). In the infarct border zone, Cx43 is redistributed along the longitudinal membrane of myocytes that border the infarct. Regionally, fibroblasts express Cx43, which occasionally is positioned at points of contact with surrounding myocytes (arrowheads; from Reference [13]). Scale bars are 20 μm in A, C, and D, and 10 μm in B. M indicates myocyte; F, fibroblast.

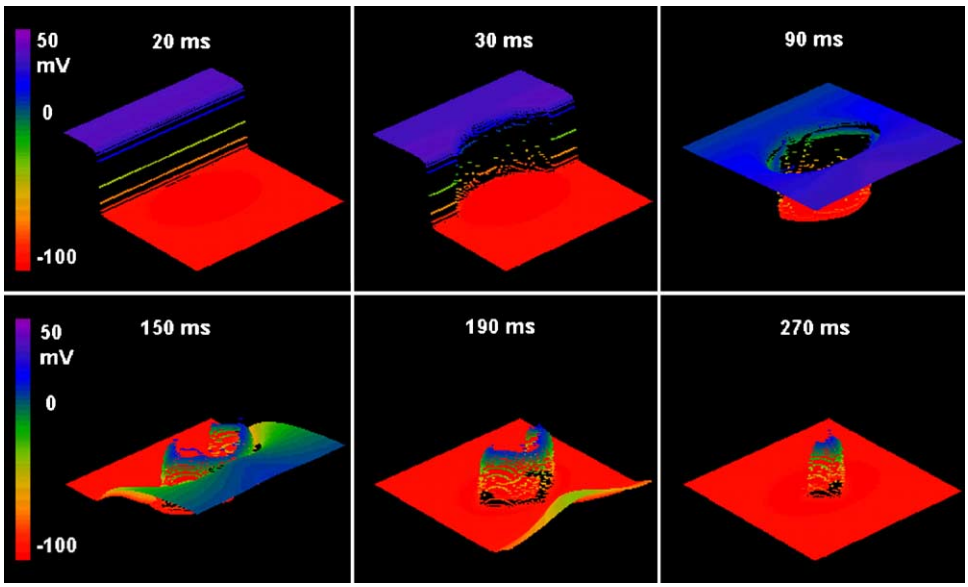

Fig. 2. Two-dimensional mathematical simulation of unidirectional block caused by regional fibrosis. The lattice of 128 × 128 cardiomyocytes contains a centrally located oval "scar" (90 × 60 cells) where cardiomyocyte coupling is reduced and each myocyte is electrically connected to 1 fibroblast. The electrical load in the area of fibrosis prevents orthograde (left-to-right) invasion of the advancing planar wave front into the scar (transmembrane potential is color- and altitude-coded, see scale bars at left). The planar wave of excitation splits, circumvents the scar, and because of the increased electrical load afforded by the "depth" of colliding wave fronts near the distal edge of the scar, invades the fibrotic tissue retrogradely (right-to-left), thereby reversing the main direction of impulse transmission. This unidirectional block will, in the case of a sufficiently large excitable gap (depending on factors such as relative and absolute speed of conduction, scar geometry, and dimensions), cause reentry of excitation.

approximation of cardiac structure and function [8]. Nonetheless, these findings confirm that the heterogeneous cell types are able to form functional electrical connections in principle. This would be of relevance for cardiac conduction if it occurred in situ.

1.3. Fibroblast-myocyte coupling in situ

Indirect electrophysiologic data that are compatible with the idea of electrical coupling of cardiac fibroblasts and myocytes in situ were first reported about a decade ago [9]. This line of research had a problem related to the high membrane resistance (GΩ) of cardiac fibroblasts: only fibroblasts that are electrically isolated from cardiomyocytes can be electrophysiologically identified with any degree of confidence in situ. Fibroblasts that are well coupled and, hence, of interest in the given context will display transmembrane potential dynamics that follow closely the action potential shape of connected cardiomyocytes. This has been confirmed both in vitro [5] and in vivo [9], making it difficult to identify fibroblasts in native cardiac tissue (lack of visual control).

A region of the heart that is particularly high in connective tissue content, even under normal conditions, is the sinoatrial node (SAN). Consequently, the SAN became a first target for dedicated histologic investigations into fibroblast-myocyte coupling in the heart. An initial transmission electron microscopic study into the morphologic substrate underlying heterogeneous cell coupling in native rabbit SAN found only a single nexuslike structure [10]. In contrast, there were abundant occurrences of heterogeneous cell contacts where fibroblast processes anchored directly into the basal membrane of adjacent cardiomyocytes [10]. Whether these regions contain dispersed gap junctional channels that are either too few or not clustered densely enough to form an electron microscopically identifiable substrate remained a subject of contention until very recently [11].

Using a combination of immunohistochemical and dye-coupling techniques, it was confirmed in 2004 that rabbit SAN tissue contains functional gap junctions, connecting cardiac fibroblasts to other fibroblasts and/or myocytes [12]. In contrast to cell cultures where heterogeneous cell coupling is dominated by connexin 43 (Cx43), in situ coupling is by connexin 40 (for fibroblast-fibroblast connections) and by connexin 45 (Cx45) (between fibroblasts and myocytes).

Current research focuses on identifying heterogeneous coupling in ventricular tissue. First pilot data suggest that such coupling occurs both in normal myocardium (Fig. 1C) and infarcted tissue (Fig. 1D). Further research is underway to quantify this coupling and to evaluate functionality of heterogeneous gap junctional channels in the ventricles [13].

2. Relevance for cardiac propagation

2.1. Connective tissue as an obstacle

The classic role of excess connective tissue as a passive barrier to impulse conduction remains, of course, a most important contributor to disturbances in cardiac propagation

[14]. The acellular component of connective tissue, in particular, can act to separate and insulate electrically excitable tissue, a process associated with several disease conditions that are linked to regional or systemic fibrosis and aging [15]. Effects of fibrosis and tissue remodeling could be confounded by an additional relative or absolute reduction in fibroblast numbers, as recently described for the aging murine heart [16].

2.2. Fibroblasts as a current sink

In addition to the passive obstruction of conduction, connective tissue cells coupled to cardiomyocytes may act as a current sink and affect myocardial excitability. In this capacity, fibroblasts could slow the generation of intrinsic excitation in the SAN (perhaps as in bradycardic sick sinus syndrome) or contribute to reduced atrioventricular conduction

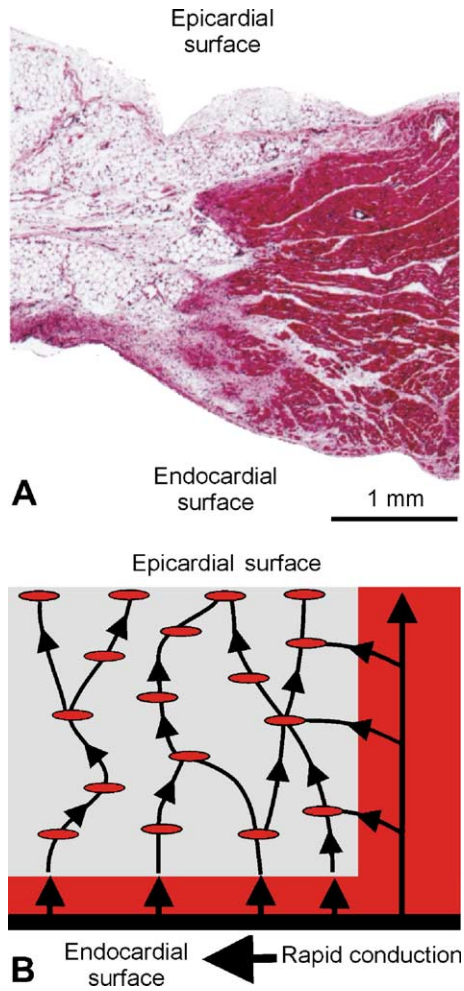

Fig. 3. Infarct structure in the rabbit heart. A, Cross section of a transmural infarct and adjacent surviving myocardium in left ventricular free wall of a rabbit heart, 8 weeks after ligation of the left descending coronary artery (hematoxylin and eosin stain). Note the intact endocardial rim and sparse myocyte groups throughout the infarct. B, Diagrammatic representation of the infarct structure and possible routes of electrical activation (arrows). Note the presence of rapid activation of the endocardial surface (via Purkinje system) and the proposed conduction between islands of myocytes via intervening fibroblasts.

velocity. In ventricular tissue, fibroblast-based current sinks could cause unidirectional block of conduction, as illustrated in the simulation shown in Fig. 2 [17]. Depending on remodeling dynamics, disease progression, and environmental parameters (such as mechanical load, heart rate, autonomic status), these effects could be either permanent or temporary, giving rise to intermittent "functional" block. Interestingly, fibroblast numbers and function can be differentially affected by otherwise homologous drugs [4]; thus, the outcome of pharmacologic interventions may be affected by (or secondary to) actions on an unintended target.

2.3. Fibroblasts as short-range conductors

Pacemaker cells in the center of the SAN show irregular orientation and are grouped in clusters, separated by patches of connective tissue [12]. Likewise, cardiomyocytes in the ventricular wall are arranged in sheets, 3 to 5 cells thick, that are separated by clear extracellular "voids" bridged by cardiac fibroblasts [18]. In both settings, fibroblasts could play important roles by connecting individual clusters of pacemaker cells in the SAN or by smoothing transmural ventricular propagation, which, given the highly anisotropic laminar substrate of the ventricular wall, would otherwise give rise to rather fractionated conduction patterns [19].

2.4. Fibroblasts as long-range conductors

Recipient-to-donor heart electrical coupling is observed after about 10% of heart transplantation cases [20]. This conduction pathway must cross scar tissue. Scars are, of course, neither static nor dead tissue [21], but in the absence of cardiomyocyte proliferation in adult heart, coupling across connective tissue barriers is likely to be based on other cells, probably fibroblasts. Similarly, ventricular infarcts often contain islands of morphologically normal cardiomyocytes. In the absence of myocyte bridges between bulk myocardium and island myocytes, functional integration into cardiac electrical behavior (which would be a prerequisite for their preserved morphologic appearance) is likely to be via fibroblasts [13].

This hypothesis is strongly supported by the recent observation of electrical impulse conduction into transmural scar tissue (Fig. 3, [22]), which has all the telltale signs of electrical conduction occurring, at least in part, via cardiac fibroblasts.

The study used an 8-week postischemic transmural infarction rabbit model [22,23]. The infarct occupies 30% to 40% of left ventricular mass and is characterized by a continuous subendocardial layer of surviving myocytes (<10% of wall thickness) and a discontinuous narrow rim of subepicardial myocytes (Fig. 3A), similar to the histopathologic appearance of human transmural infarcts [24]. Scar tissue contained fibroblasts that surround scattered sparse islands of surviving myocytes.

Despite this heterogeneous structure, optical mapping of epicardial electrical activity (using voltage sensitive dyes) revealed relatively normal activation patterns during atrial

pacing [22,25]. The thickness of the transmural scar tissue (millimeters) makes it unlikely that the epicardially recorded electrical activity originates from the subendocardial layer of preserved myocardium. The presence of regular action potential–like epicardial electrical activity on the surface of the transmural infarct suggests that either (1) the remaining scattered cardiomyocytes in the scar tissue are electrically integrated into the physiological sequence of activation, presumably via fibroblasts, or (2) that fibroblasts, connected to cardiomyocytes, mimic cardiac action potentials sufficiently well to serve as a source for the optical signal (or that both of these possibilities apply). In each case, cardiac fibroblasts would intrinsically be involved in electrical impulse transmission in the heart.

Fig. 3B schematically illustrates a possible scenario of electrical invasion of a transmural scar, based on predominant conduction in a subendocardial rim, potentially containing surviving Purkinje cells, as observed in human myocardium [24,26].

3. Outlook

Thus far, functional coupling of individual cardiac fibroblasts and myocytes has not been confirmed in situ outside the SAN. Indirect electrophysiologic evidence suggests that heterogeneous coupling may be present in atrial tissue and, judging by preliminary immunohistochemical findings and optical mapping of impulse conduction into transmural scars, fibroblast myocyte coupling may also be present in normal and diseased ventricular myocardium.

The histologic substrate for heterogeneous cell coupling is likely to vary regionally (and/or with species), with Cx45 in the rabbit SAN, Cx43 in healthy sheep ventricle, and Cx43 or Cx45 in sheep ventricular scars. In addition, at least in pathological states, fibroblast connexin expression would appear to be subject to dynamic remodeling, with 2 clearly distinct populations involved in postmyocardial scar development [13].

An interesting further aspect of cardiac fibroblast function is their mechanosensitivity, not only in the context of gene expression, protein synthesis, extracellular matrix turnover, or auto- and paracrine function, but also directly in electrophysiologic terms. Fibroblasts possess stretch-activated ion channels, and fibroblast membrane resistance and potential change considerably with mechanical stimulation [27]. Thus, fibroblasts coupled to myocytes will affect cardiac electrophysiology differently, depending on acute mechanical loading conditions.

Furthermore, fibroblasts may affect therapeutic outcomes of interventions that target cardiac mechanics, electrophysiology, or tissue structure. In this capacity, they could be an interesting drug target or a cause of unwanted side effects. In fact, the question as to whether one would want to increase, or reduce, the electrical coupling between heterogeneous cardiac cell populations will depend on the actual pathophysiologic context. Interestingly, drugs or vectors that target fibroblast ability to interact with the surrounding cardiomyocytes are becoming experimental reality [28].

Further studies are required to identify the electrophysiologic relevance of cardiac fibroblasts in situ and how age, disease, drugs, and environmental variables affect this interaction. This will be of fundamental interest for an integrative understanding of cardiac electrical function and for the optimization of existing, or identification of novel, therapeutic concepts.

Acknowledgments

This study was supported by the UK Biotechnology and Biological Sciences Research Council (Swindon, UK) and the British Heart Foundation (London, UK). We thank Dr Anthony Varghese, University of Minnesota, for his mathematical modeling work underlying Fig. 2.

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